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THE INFLUENCE OF THE X-RAY ON THE PRODUCTION OF ANTIBODIES *

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Heinecke and others[1] have shown that in proper doses the x-ray has a direct and destructive action on the lymphoid and myeloid tissues. The destruction begins very soon after the exposure; the lymphatic glands and follicles appear to be affected first, next the spleen, and then the bone-marrow. The lymphocytes in the thymus are quickly destroyed by the x-ray.[2] The cells undergo direct necrosis with nuclear fragmentation, and, if the process is made to take place gradually, destruction of the lymphoid cells and the myelocytes may be accomplished without appreciable effect on other tissues or on the general health of the animals. The leukocytes in the circulating blood are reduced in number, and, when this effect is marked, the mononuclears proportionately more than the polymorphonuclears.[3] When the action of the ray is at an end, regeneration of cells and reconstruction take place.

In view of this action on the hematopoietic system, the question arises what effect has the x-ray on the production of antibodies? This question has received some attention and attempts have been made to study it along two principal lines, namely, by the direct study of the development of antibodies in animals treated with the x-ray and by observations on the resistance of x-rayed animals to infection. Benjamin and Sluka[4] found that in rabbits exposure to the x-ray before the injection of beef serum diminished very much the production of specific precipitin or prevented its formation altogether at the same time as there resulted, in some cases, a considerable delay in the disappearance of the antigen from the blood of the injected animals. If applied four days or so after the injection of beef serum, or at the

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[1] Heinecke, Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1905, 14, p. 21; Warthin, Internat. Clin., 1905, 4, p. 243; Murphy and Ellis, Jour. Exper. Med., 1914, 20, p. 397.

[2] Rudberg, Thymusinvolution efter Röntgenbestrålning, Upsala, 1909.

[3] Concerning changes in blood in workers with the x-ray, see Portis, Jour. Am. Med. Assn., 1915, 65, p. 20.

[4] Wien. klin. Wchnschr., 1908, 21, p. 311.

height of precipitin production, however, the ray was without any effect on the precipitin. Läwen[5] observed that the formation of specific bacterial agglutinin and lysin was restrained in animals treated with the x-ray, and that no action was had on agglutinin in the blood of normal animals or on typhoid agglutinins subjected to exposure in vitro.

Von Heinrich[6] studied the effects of the x-ray on the phenomena of anaphylaxis in guinea-pigs. He found that animals given a single exposure of x-ray ("3 Kalom, 1 Erythemdosis") soon after the primary injection of antigen, reacted very much less violently on re-injection of the antigen about fourteen days later than did animals which were not exposed to the x-ray. Furthermore, passive anaphylaxis could not be produced with the serum of guinea-pigs treated with the x-ray soon after injection of antigen; hence, it would seem logical to describe the action of the x-ray as interference with the production of the specific body on which anaphylactic shock depends. This view is supported by the fact that the x-ray appears to have no effect on the complement. In animals that received the second injection some six weeks after the primary injection, which had been followed by exposure to the x-ray, the reactive phenomena are described as especially severe. Von Heinrich suggests that the antigen may have remained in available form so long that when regeneration of the blood-making tissues set in after the x-ray injury, specific antibody was produced. This explanation is in accord with the observation of Benjamin and Sluka that the antigen persists longer in the blood of rabbits treated with the x-ray than in the blood of rabbits not so treated.

Buckner[7] tried to stimulate, by means of exposures to x-ray, the production of specific antibodies in mice injected with trypanosome vaccine, but the results obtained were not constant.

Of the experiments designed to test the effect of the x-ray on the resistance of animals to infection, the earliest ones were based on the idea that, on destruction of leukocytes by the x-ray, substances would be set free that would protect the animal.[8] Under the conditions of certain experiments, indications of increased resistance were obtained, but as yet we have no confirmatory reports. On the other hand, Läwen was unable to detect any changes in the blood serum or any setting free of endoleukocytic enzymes after destruction of leukocytes by means of the x-ray, and he did find that the resistance to various bacteria on the part of rats, mice, and guinea-pigs was reduced greatly thereby. More recently, Murphy and Ellis[9] find that after exposure to the x-ray mice (normal and splenectomized) became more susceptible to bovine tuberculosis than normal animals. X-ray, in the doses they used, affected only lymphoid tissue, and this result in conjunction with other facts leads them to regard the lymphocyte as an important agent of defense against tuberculosis.

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[5] Mitt. a d. Grenzgeb. d. Med. u. Chir., 1909, 19, p. 141.

[6] Centralbl. f. Bakteriol., I, O., 1913, 70, p. 421.

[7] Ztschr. f. Hyg. u. Infektionskrankh., 1913, 73, p. 411.

[8] Quadrone, Centralbl. f. in. Med., 1905, 26, p. 521; Heile, Ztschr. f. klin. Med., 1904, 55, p. 508; Helber and Linser, München. Med. Wchnschr., 1905, 52, p. 689.

In connection with other experimental work on the production of antibodies under various conditions, it has been found desirable to study the effect of the x-ray also. For this purpose, the white rat has been used, and the antibody observed is the specific lysin that develops after the injection of sheep blood. The exposures to the x-ray have been made in the x-ray laboratory of the Presbyterian Hospital of Chicago and I am under much obligation to Dr. Hollis E. Potter and Dr. J. W. Rowntree.

Experiment 1.—Twenty-five rats, of about the same age, weighing from 60 to 80 grams were used.

The distance from the tube to the rats was 18 inches. Secondary current, 4 milliamperes. Tube backing up 5 inches parallel spark-gap. Time of each exposure, eight minutes.

Immediately after the first exposure, the rats were injected intraperitoneally with 5 c.c. per kilo of weight of a 10 percent suspension of sheep blood. Subsequently, the rats were given the same exposure to x-ray daily. After the second day, two rats were killed every day or every other day, the leukocytes counted, and the titer of the lysin for sheep corpuscles in the serum determined. After the fifteenth day, only one rat was killed at a time. In no case did the general health seem to be affected. The thymus especially and the spleen were usually diminished in size and often the lymph nodes were reduced to mere traces.

The determination of the lysin was made by finding the highest dilution of the serum, heated at 56 C. for thirty minutes, and complemented with guinea-pig serum (0.0125 c.c.), at which distinct lysis was produced. The total quantity in each tube was 0.6 c.c., including 0.2 c.c. of a 5 percent suspension of sheep corpuscles, carefully washed, 0.0125 c.c. of guinea-pig serum, and the requisite amount of heated rat serum, the rest being salt solution. The mixtures were incubated for two hours and then placed in the ice-box until the following morning.

The results of the measurements of the lysin and the leukocyte counts are given in Table 1. The figures under "Controls" give the titer of the lysin of twenty-five rats of the same age and weight, treated in exactly the same way as the x-ray rats, except that they were not exposed to x-rays. In all cases, the figures under "Lysin" represent the highest dilution of the serum in which definite lysis occurred.

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[9] Jour. Exper. Med., 1914, 20, p. 397.

TABLE 1

THE LYSIN AND LEUKOCYTES IN EXPERIMENT 1 AND THE LYSIN IN THE CONTROL RATS

Days After First Exposure and Injection of Antigen	X-ray Rats				Lysin in Controls	
	Lysin		Leukocytes			
2	96	96	2,200	1,800	192	192
4	1,536	384	2,800	2,550	6,144	12,288
5	6,144	6,144	2,450	2,600	12,288	12,288
6	3,072	6,144	2,400	2,050	12,288	6,144
7	12,288	3,072	2,100	2,000	6,144	3,072
8	1,536	192	2,100	2,050	12,288	6,144
9	1,536	12,288	1,850	1,900	768	384
10	1,536	3,072	1,800	1,800	1,536	6,144
12	3,072	1,536	1,800	1,650	3,072	3,072
15	768	768	1,700	1,500	1,536	1,536
16	192	......	1,600	.....	768	......
17	384	......	1,552	.....	768	......
18	192	......	1,600	.....	192	.....
20	48	......	.....	.....	192	......
22	48	......	.....	.....	192	......

TABLE 2

THE LYSIN AND LEUKOCYTES IN EXPERIMENT 2

Days After First Exposure	Days After Injection of Antigen	Lysin		Leukocytes	
3	2	384	384	3,600	3,150
4	3	384	384	2,350	2,700
5	4	384	384	2,000	1,750
6	5	12,288	1,536	2,100	.....
7	6	12,288	12,288	1,800	2,050
8	7	12,288	12,288	2,700	2,100
10	9	12,288	3,072	1,650	1,800
11	10	1,536	6,144	1,500	1,450
12	11	1,536	......	1,450	.....
14	13	1,536	384	1,050	1,400
17	16	192	384	1,300	1,150
19	18	384	......	950	

The results show a prompt reduction in the number of leukocytes in the rats exposed to the x-ray, differential counts showing the polymorphonuclear leukocytes to be relatively increased. The results do not indicate, however, that the exposures restrained in any noteworthy degree the formation of lysin in the x-ray rats as compared with the controls. Possibly the formation of lysin was checked somewhat in the beginning in the x-ray animals, and the figures point also to a more rapid fall in them, but the differences are hardly marked enough not to be explainable by individual variations and errors of observation. As the figures for each day represent the rats killed that day, individual variation must be considered in judging of the results.

Experiment 2.—In this experiment, twenty-two rats, of about the same age, weighing from 60 to 80 grams each, were exposed to x-ray under the same general conditions as in Experiment 1, but the length of each exposure was twice as long as in the first experiment, that is, sixteen minutes. The injection of sheep blood was made after the second exposure. The results are given in Table 2.

In this experiment, also, the results do not indicate that the effects of the x-ray in any considerable degree restrained the production of lysin, even tho the number of leukocytes was greatly reduced. On comparing the amounts of lysin in the serum of these rats with the amounts in the serum of the control rats in Experiment 1, it appears that at least during the first five days the lysin production remained considerably below that in the control animals.

In the next experiment, the rats were brought thoroughly under the influence of the x-ray before injecting the antigen.

Experiment 3.—In this experiment, nineteen young rats were x-rayed as in Experiment 1 for ten days before sheep corpuscles were injected. In the meantime, the leukocytes ranged around 3,000-4,000. The exposures were then continued, one rat being killed each day. In this series, also, the exposures were without any noticeable effect on the general condition of the rats. The results are given in Table 3.

TABLE 3

The Lysin and Leukocytes in Experiment 3

Days After First Exposure to X-ray	Days After Injection of Sheep Blood	Lysin	Leukocytes
11	1	96	2,100
12	2	192	
13	3	96	2,250
14	4	96	2,100
16	6	96	2,200*
17	7	3,072	2,550
18	8	3,072	1,800
19	9	768	2,400
20	10	96	2,550**
21	11	1,536	1,750
23	13	48	1,800
24	14	96	1,450
25	15	48	1,950***

* Granulocytes, 80%; lymphocytes, 20%.
** Granulocytes, 53%; lymphocytes, 47%.
*** Granulocytes, 78%; lymphocytes, 22%.

This result was practically duplicated in Experiment 4, in which the ticed for some days before the introduction of antigen and then continued, show that the formation of lysin was restrained very markedly. It is noteworthy that the total number of leukocytes is not depressed any more than in Experiments 1 and 2; the relative decrease of mononuclears is marked, however.

This result was practically duplicated in Experiment 4, in which the exposure to the x-ray was not continued so long as in Experiment 3.

EXPERIMENT 4.—In this experiment, sixteen young rats were x-rayed as in Experiment 1 for five days and then given a double exposure for five days, sheep blood being injected intraperitoneally on the eighth day from the beginning. Two rats were killed each day from the second day after injection of sheep blood to the thirteenth inclusive; the three remaining animals were killed as shown in Table 4.

TABLE 4

THE LYSIN AND LEUKOCYTES IN EXPERIMENT 4

Days After First Exposure to X-ray	Days After Injection of Sheep Blood	Lysin	Leukocytes		
			Total	Granulocytes	Lymphocytes
8	2	24	1,400	44	56
		0	1,500	66	34
9*	3	0	900	40	60
		24	600	50	50
10	4	0	2,000	50	50
		48	700	40	60
11	5	96	700	40	60
		96	1,100	50	50
12	6	0	1,000	55	45
		0	1,000	55	45
13	7	24	1,100	56	44
		384	1,450	52	48
14	8	768	1,400	40	60
15	9	768	1,300	40	60
17	11	768	1,900	34	66

* Last exposure to x-ray.

In the rats of Experiment 4 examined before the seventh day after the injection of sheep blood, the spleen was very small, the thymus greatly reduced, and the lymph glands apparently absent, the bone-marrow being softer and more deeply red than normally. In the last three rats, the spleen was not so small and the thymus showed distinct islands of regeneration. The presence of newly formed lysin in the rats killed on the fourteenth, fifteenth, and seventeenth days may be accounted for by assuming that in these rats lysin production was not restrained very much and that when killed the course of the lysin had passed the high point on the return to normal conditions; or we may assume that the lysin was the result of more recent activity by the centers of antibody production, antigen (sheep blood) remaining in available form until the centers had recovered from the effects of the x-ray. The second suggestion coincides with v. Heinrich's explanation of the severe anaphylactic reaction in guinea-pigs six weeks or so after the primary injection, followed by treatment with x-ray.

EXPERIMENT 5.—This experiment illustrates the result from a single exposure of twenty minutes, 10 milliamperes through Coolidge tube, focal distance 7 inches, spark-gap 5 to 6 inches, the antigen being injected immediately afterwards, each animal being bled from the heart on the fourth day or so and thereafter at intervals of two to three days while alive. Most of the rats so treated would die in eight to ten days with the usual symptoms of profound x-ray effects and as a rule with no, or very little, new production of lysin, the number of leukocytes being reduced very much, in some cases 400-500. In the animals that survived longer, some little lysin might appear in the blood after a longer period of latency than was observed in rats not exposed to x-ray.

TABLE 5

THE LYSIN AND LEUKOCYTES IN RATS EXPOSED TO X-RAY FOR TWENTY MINUTES BEFORE INJECTION OF ANTIGEN

Days After Injection of Antigen	Rat 1		Rat 2		Rat 3		Rat 4	
	Lysin	Leukocytes	Lysin	Leukocytes	Lysin	Leukocytes	Lysin	Leukocytes
4............	48	—	48	1,500	0 (24)	1,150	0 (24)	—
6............	48	3,000	192	500	48	400	96	800
8............	48	—	1,536	1,100	96	600	192	
10............	Died	.....	Died	.....	Died			
11............	.....	.....	.....	.....	.....	.....	1,536	
12............	.....	.....	.....	.....	.....	.....	Died	

In this experiment, some of the animals showed no indication of lysin formation for eight days after the injection of the antigen; in others, there resulted production of antigen after a much longer period of latency than was the case in normal rats (see Table 1).

In the serum of the rats that failed to produce lysin after exposure to the x-ray, at least as judged by the tests, it is possible that inhibitory or antilytic substances were present. That such was the case does not seem likely, however, because the serum of x-rayed animals had no inhibitory effect whatever on the lysin in the serum of animals not treated with the x-ray or treated as in Experiments 1 and 2. The two kinds of serum were mixed in varying proportions and incubated for twenty-four hours, but no antilytic effect was obtained even in mixtures of one part of lytic serum to 192 parts of non-lytic x-ray serum.

Experiments were made to test the effect of the x-ray on the course of lysin in rats after its production was well under way. The results obtained are not conclusive. In order to study this point satisfactorily, it is necessary to bleed the same animal several times and this is not possible in the case of rats which have been exposed freely to the x-ray, as they do not stand repeated punctures of the heart. For this reason, further consideration of the effect of the x-ray on the course of antibodies is postponed until it can be studied in a larger animal and with reference also to other antibodies.

SUMMARY

Repeated exposures of white rats to the x-ray immediately after the injection of sheep blood do not interfere in any noteworthy degree with the production of lysin when the exposures, tho sufficient to reduce the number of leukocytes in the blood, have no definitely detrimental effect on the general health of the animals.

When the exposures are commenced some days before the injection of sheep blood and continued in such manner that the number of leukocytes is greatly reduced for fifteen to twenty days after the injection of sheep blood, the formation of lysin is restrained to a marked degree. This may be accomplished without any appreciable effect on the general health. In such animals the spleen, lymphatic tissues, and thymus are greatly reduced in volume and the bone-marrow changed. In the blood, the granular leukocytes are increased relatively and the total number of leukocytes is much below normal.

A single prolonged and eventually fatal exposure to the x-ray immediately before the injection of sheep blood may prevent any formation of lysin for the next eight days or so, or lysin may be produced after a longer period of latency than in the control animals.

The results harmonize with the view that antibodies are produced in the spleen, lymphatic tissues, and marrow, as these structures suffer most directly from the action of the x-ray. The results indicate also that one reason why the lymphocyte appears to be an important agent of defense in tuberculosis and other conditions may be its power to form antibodies.

In view of the destructive action of radium on the leukocytes and bloodmaking organs, it is altogether likely that it will be found to have the same general effect on the production of antibodies as the x-ray.